IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of	:	**Mail Stop: APPEAL BRIEF-PATENTS**
Keiko YAMASAKI et al.	:	**Confirmation No. 3583**
Serial No.09/914,265	:	Docket No. 2001-1026A
Filed September 5, 2001	:	Group Art Unit 1615
EXTERNAL SKIN PATCH	:	Examiner Isis GHALI

APPEAL BRIEF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

THE COMMISSIONER IS AUTHORIZED TO CHARGE ANY DEFICIENCY IN THE FEES FOR THIS PAPER TO DEPOSIT ACCOUNT NO. 23-0975

Sir:

This is an appeal from the final decision of the Examiner set forth in the Office Action dated June 30, 2004, finally rejecting claims 7-11, which are attached herewith in the Claims Appendix. A Petition requesting a one-month extension of time accompanies this brief. A Notice of Appeal was filed on December 30, 2004.

TABLE OF CONTENTS

PAGE

I. REAL PARTY IN INTEREST

The real party in interest is Teikoku Seiyaku Co., Ltd., assignee of the entire right, title and interest to this application.

II. RELATED APPEALS AND INTERFERENCES

There are no related prior nor pending appeals, interferences, or judicial proceedings known to Appellants, Appellants' legal representatives, or assignee which will affect or be affected by, or have a bearing on the Board's decision in the present appeal.

III. STATUS OF CLAIMS

The status of the claims as indicated in the Advisory Action dated January 19, 2005 is as follows:

Claims pending:	7-11
Claims canceled:	1-6 and 12
Claims withdrawn:	none
Claims rejected:	7-11
Claims appealed:	7-11

IV. STATUS OF AMENDMENTS

The last amendment to the claims was in the after final response filed on December 30, 2004. In item 7(b) on page 1 of the Advisory Action dated January 19, 2005, it is indicated that this amendment will be entered for purposes of Appeal.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

A. Independent Claim 7

The invention of independent claim 7 is a novel external skin patch comprising a substrate and a drug reservoir layer coated on the substrate, in which the drug reservoir layer

comprises (1) an adhesive gel base which comprises a water soluble polymeric material, a crosslinking agent, 20 to 60% by weight of water based upon the total weight of the adhesive gel base and a humectant as essential components, together with (2) medicinal components comprising a local anesthetic and a nonsteroidal antiphlogistic analgesic agent (specification, page 5, lines 1-10).

The external skin patch according to the present invention has a substrate and a drug reservoir layer coated on the substrate (specification, page 13, lines 4-25).

The substrate includes such substances as polyester, polyvinyl chloride, lint, nylon, an unwoven fabric or a composite material thereof (specification, page 6, lines 12-17).

The drug reservoir layer of the external skin patch comprises a drug-containing base comprising an adhesive gel base and a local anesthetic and a nonsteroidal antiphlogistic analgesic agent as medicinal components (specification, page 7, lines 1-5).

The adhesive gel base employed according to the present invention contains a water soluble polymeric substance, a crosslinking agent, water and a humectant as essential components (specification, page 7, lines 7-10).

The water soluble polymeric material of the adhesive gel base includes substances, such as gelatin, starch, agar, mannan, alginic acid, polyacrylic acid, a salt of polyacrylic acid, dextrin, methyl cellulose, hydroxypropyl cellulose, methyl cellulose sodium, carboxymethyl cellulose, carboxymethyl cellulose sodium, polyvinyl alcohol, polyvinyl pyrolidone, methyl vinyl ether-maleic anhydride copolymer, gum Arabic, gum tragacanth, karaya gum, locust bean gum, and the like (specification, page 7, lines 11-18).

The crosslinking agent of the adhesive gel base is selected from aluminum compounds (specification, page 8, lines 6-9).

The humectant of the adhesive gel base is selected polyhydric alcohols, saccharides or superabsorbent resins (specification, page 9, lines 7-17).

The water content of the adhesive gel base is 20 to 60% by weight of water based upon the total weight of the adhesive gel base (specification, page 9, lines 1-6).

The local anesthetic of the drug reservoir layer is tetracaine, procaine, dibucaine, benzocaine, xylocaine or pharmaceutically acceptable salts thereof (specification, page 10, lines 3-8).

The nonsteroidal antiphlogistic analgesic agent of the drug reservoir layer is ketoprofen, piroxicam, felbinac, bufexamac, suprofen, flurbiprofen, ibuprofen or pharmaceutically acceptable salts thereof (specification, page 10, lines 18-23).

The external skin patch exhibits improved antiphlogistic and analgesic painkilling effects for pains accompanied by inflammation, such as chronic arthrorheumatism, arthrosis deformans or low back pain (specification, page 4, lines 9-12).

B. Dependent Claims 8-11

The inventions of dependent claims 8-11 relate to the external skin patch containing a specific local anesthetic and a specific nonsteroidal antiphlogistic analgesic agent in certain amounts.

In particular, the inventions of claims 8 and 9 relate to the external skin patch wherein the local anesthetic and the nonsteroidal antiphlogistic analgesic agent are present in an amount of 0.1-50% and 0.05-10% by weight, respectively (specification, page 5, line 24 to page 6, line 6).

The inventions of claims 10 and 11 relate to the external skin patch wherein the local anesthetic is benzocaine or a pharmaceutically acceptable salt thereof, and the nonsteroidal antiphlogistic analgesic agent is ketoprofen, felbinac, flurbiprofen, or a pharmaceutically acceptable salt thereof (specification, page 5, lines 11-23).

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A. Claims 7-11 were rejected under 35 U.S.C. § 103(a) as obvious over Mantelle et al., U.S. Patent No. 6,562,363 in view of Oda et al., U.S. Patent No. 5,725,874.

B. Claims 7-11 were rejected under 35 U.S.C. § 103(a) as obvious over Liedtke, U.S. Patent No. 5,686,112 in view of Oda et al., U.S. Patent No. 5,725,874.

VII. ARGUMENT

A. Rejection Under 35 U.S.C. § 103 – US '363 in view of US '874

Claims 7-11 were rejected under 35 U.S.C. § 103(a) as obvious over Mantelle et al., U.S. Patent No. 6,562,363 ("US '363") in view of Oda et al., U.S. Patent No. 5,725,874 ("US '874"). See items 1 and 3 on pages 2-5 and 9 of the final Office Action dated June 30, 2004 and page 2 of the Advisory Action dated January 19, 2005.

This rejection is respectfully traversed for the following reasons.

To establish obviousness, three criteria must be met. First, the prior art references must teach or suggest each and every element of the claimed invention. See In re Royka, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974); In re Wilson, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494, 496 (C.C.P.A. 1970); M.P.E.P. § 2143.03. Second, there must be some suggestion or motivation in the references to either modify or combine the reference teachings to arrive at the claimed invention. See In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991); M.P.E.P. § 2143.01. Third, the prior art must provide a reasonable expectation of success. See In re Merck & Co., Inc., 800 F.2d 1091, 231 U.S.P.Q. 375 (Fed. Cir. 1986); M.P.E.P. § 2143.02.

i. Independent Claim 7

Independent claim 7 calls for an external skin patch which comprises, among other ingredients, ***20 to 60% by weight of water based upon the total weight of the adhesive gel base***.

US '363 and US '874 fail to render obvious the claimed invention, because they fail to disclose and/or suggest an external skin patch with this claimed water content.

US '363 relates to a topical bioadhesive composition comprising two or more active agents including a non-steroidal anti-inflammatory, a local anesthetic and an analgesic (column 8, lines 24-29; column 50, claim 15). At lines 16-19 on page 4 of the final Office Action, it is

argued that all elements of the claimed composition are taught by US '363 except for the specific cross-linking agent.

However, it is respectfully submitted that US '363 discloses nothing about an external skin patch having a layer containing an adhesive gel base comprising 20% to 60% by weight or more of water as an essential component. Thus, in contrast to the indication by Office, US '363 does ***not*** disclose or suggest "all elements" of the claimed composition.

Likewise, US '874 fails to disclose or suggest an external skin patch comprising 20 to 60% by weight of water based upon the total weight of the adhesive gel base. As noted at lines 16-19 on page 4 of the final Office Action, US '874 is relied upon ***solely*** for teaching the use of aluminum compounds as crosslinking agents.

Thus, US '363 and US '874 do not disclose or suggest each and every element of the claimed invention, namely, an external skin with the claimed water content.

Furthermore, there is no motivation to combine and/or modify the references to arrive at the claimed skin patch, because US '874 teaches nothing about a composition comprising a polyvinylpyrrolidone polymer as described in US '363. Also, as will be discussed further below, the formulation of US '363 is substantially-free water, and thus, it is entirely different from the composition of US '874. As such, there is no suggestion and/or motivation to combine their teachings to arrive at the claimed invention.

Moreover, US '363 actually ***teaches away*** from a pharmaceutical composition containing 20% to 60% by weight or more of water. Consequently, the references ***lack the requisite motivation to combine and/or modify*** their teachings to arrive at the claimed invention. In other words, they cannot be combined to arrive at the claimed invention.

It is well established that the cited prior art must be considered in its entirety, including disclosures that teach away from the claimed invention. See W.L. Gore & Associates, Inc. v. Garlock, Inc., 721 F.2d 1540, 220 U.S.P.Q. 303 (Fed. Cir. 1983), cert. denied, 469 U.S. 851 (1984); M.P.E.P. § 2141.02..

Moreover, references cannot be combined where the references teach away from their combination. See M.P.E.P. § 2145 X, D, 2. A reference can be said to teach away when a person of ordinary skill in the art, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path taken by the applicant. Ibid.

In this case, at column 7, lines 35-44 of US '363, it is disclosed that:

> An important characteristic of the embodiments of the present invention relates to the ***substantially water-free*** and ***water-insoluble nature*** of the composition. By the term "substantially water-free" is meant that the composition contains ***less than about 10% by weight water***, and preferably less than 5%, and most preferably less than 3% prior to its topical application. In general, it is ***desirable to avoid the addition of water entirely*** and ***to eliminate, as far as possible, the presence of water*** in the other ingredients of the composition. [Emphasis added.]

Based on such disclosure, it is clear that the pharmaceutical formulation of the composition of US '363 is a ***substantially water-free film***. See also column 7, lines 35-45; and column 49, claim 1 of US '363. In other words, US '363 teaches away from a pharmaceutical composition containing water, because it teaches away from the inclusion of water in the composition.

Upon reading US '363, one of ordinary skill in the art would be led in a path ***divergent*** from that taken by the Applicant. Consequently, there is no motivation to combine the teaching of US '363 with that of US '874 to arrive at the claimed invention. US '363 teaches away from the inclusion of water (less than 10%), and thus, US '363 cannot be combined with US '874 to arrive at the claimed invention which is directed to 20 to 60% by weight of water.

In addition, US '363 describes that "this invention relates to compositions capable of being used in wet or moist environments, especially on mucous membranes, for a prolonged period of time." See column 1, lines 13-16 of US '363. This means that the composition of US '363 is intended to be mainly applied to mucous membranes, as opposed to the skin as in the present invention. As such, it is not at all clear that the bioadhesive composition in US '363 is even an external skin patch. Therefore, the bioadhesive composition of US '363 is essentially

different from the base composition of the external skin patch of the present invention which is an adhesive gel base containing 20 to 60% by weight of water as an essential component.

On page 5 of the final Office Action, it was argued that the Appellants have not shown the criticality of the claimed water content.

In reply, Appellants submitted the Declaration under 37 C.F.R. § 1.132 by Mitsui Akazawa ("Akazawa Declaration") with the after final response of December 30, 2004 as evidence of the criticality of the water content of the claimed invention.

The Akazawa Declaration describes animal experiments distinguishing the external skin patch of the claimed invention containing 20-60% by weight of water content from the substantially water-free film of US '363 containing less than 10%.

In particular, Examples (A) and (B) comprise the claimed external skin patch with a water content of 60% and 20% by weight, respectively. See lines 20-21 on page 2 of the Akazawa Declaration. Such water content covers the claimed range of 20-60%. Comparative Example (A) relates to a skin patch which is substantially water-free as prepared according to Example 8 of US '363, except that the film contains the same local anesthetic and nonsteroidal antiphlogistic analgesic agent in the same amounts according to the claimed invention of Examples (A) and (B). See item 4(1) on page 2 and item 4(2) on page 3 of the Akazawa Declaration.

Examples (A) and (B) and Comparative Example (A) were topically applied to the paws of rats in an animal model to assess the animals' threshold for pain. The experimental results of the comparison are in Table III on pages 3-4. Examples (A) and (B) achieved a remarkable and unexpected increase in pain threshold of 55.8% and 53.5%, respectively. On the other hand, Comparative Example (A) only achieved an increase of 20.9%. Thus, the results of this animal study clearly demonstrate the superior pain relief effect of the present invention over a skin patch which is substantially water-free.

Based on this data, it is clear that the unexpectedly superior pain relief effect of the present invention ***cannot be achieved by the skin patch containing less than 10% by weight of***

water as called for by US '363. Thus, pursuant to the Examiner's request in the final Office Action, the Akazawa Declaration provides evidence of the criticality of the claimed 20-60% water content in the present skin patch over a prior art composition containing less than 10%.

Nonetheless, on page 2 of the Advisory Action, it is indicated that the Akazawa Declaration is unpersuasive, because: (1) no statistical evaluation was provided to determine if there is any additive or synergistic effect of both elements of the composition; and (2) the evaluation in the Declaration is subjective and based on individual judgment.

Appellants respectfully disagree with the Office's position and submit that the data in the Akazawa Declaration sufficiently establishes the criticality of the claimed water content and the unexpectedly superior pain relief effect of the present invention which cannot be achieved by the skin patch containing less than 10% by weight of water. In this regard, attached herewith is the reference, Drug Discovery and Evaluation, p 384-385, p406-409, Vogel et al. eds. (1997). This reference discloses that the experiments and analysis conducted in the Akazawa Declaration are common and accepted practices in the industry.

The attached reference is being submitted for the first time with this Appeal Brief to respond to the arguments raised for the first in the Advisory Action regarding the credibility of the data in the Akazawa Declaration.

Thus, it is respectfully submitted that the Akazawa Declaration is persuasive in that the experiments use an industry accepted animal model to distinguish the skin patch of the claimed invention containing 20-60% by weight of water content from the substantially water-free film of US '363 containing less than 10%.

In view of the above, the cited references fail to disclose or suggest each and every element of the claimed invention, and they lack motivation to apply the combination of drugs described in US '363 to the preparations disclosed in US '874 to arrive at the claimed invention of an external skin patch which comprises, among other ingredients, 20 to 60% by weight of water based upon the total weight of the adhesive gel base with an expectation of achieving a superior pain relief effect. Also, notwithstanding that the claimed invention is not obvious over

the cited prior references, the external skin patch of the present invention achieves a superior and unexpected pain relief effect that would not have been discernable to one of ordinary skill in the art upon reading US '363 and US '874, as evidenced by the Akazawa Declaration and the Declaration under 37 C.F.R. § 1.132 by Keiji Nozaki ("Nozaki Declaration"), which was submitted with the response filed May 20, 2004.

The Nozaki Declaration demonstrates that the claimed invention possesses an ***unexpected synergistic effect*** in comparison to the administration of a random combination of anesthetic or analgesic as taught in the prior art. The Nozaki Declaration will be discussed in detail below. Again, such unexpected synergistic effects are indicative of non-obviousness of the claimed invention.

For these reasons, the rejection of independent claim 7 under 35 U.S.C. § 103(a) as obvious over US '363 and US '874 is untenable and should be reversed.

ii. Dependent Claims 8-11

The inventions of dependent claims 8-11 relate to the external skin patch of claim 7, but further containing a specific local anesthetic and a specific nonsteroidal antiphlogistic analgesic agent in certain amounts.

These claims are also novel and unobvious over the prior art, since, as discussed above, the prior art fails to disclose or suggest an external skin patch comprising the claimed water content and the specific combination of a local anesthetic and a specific nonsteroidal antiphlogistic analgesic agent in the claimed amounts.

Thus, in view of above discussion regarding independent claim 7, the rejection of dependent claims 8-11 under 35 U.S.C. § 103(a) as obvious over US '363 and US '874 is also untenable and should be reversed.

B. Rejection Under 35 U.S.C. § 103 – US '112 in view of US '874

Claims 7-11 were rejected under 35 U.S.C. § 103(a) as obvious over Liedtke, U.S. Patent No. 5,686,112 ("US '112") in view of US '874. See items 2 and 3 on pages 5-9 of the final Office Action and page 2 of the Advisory Action.

This ground of rejection is respectfully traversed for the same reasons discussed above regarding US '874 and for the following reasons.

i. Independent Claim 7

Again, as noted above, independent claim 7 calls for an external skin patch comprising a specific combination of a local anesthetic and a nonsteriodal analgesic, and other ingredients, including 20 to 60% by weight of water based upon the total weight of the adhesive gel base.

US '112 and US '874 fail to render obvious the claimed invention, because they fail to disclose and/or suggest an external skin patch with these claimed elements.

US '112 is relied upon as suggesting a combination of non-steroidal anti-inflammatory analgesics and local anesthetics which are delivered in single dosage topical pharmaceutical form. See for instance page 6 of the final Office Action.

However, US '112 does not disclose the specific combination of the nonsteroidal antiphlogistic analgesics and local anesthetics of the present invention. Instead, the active compounds described in US '112 are "analgesics and local anesthetics such as buprenorphine, fentanyl, penzocaine, morphine and morphine derivatives, lidocaine, prilocaine, mepivacaine or non-steroidal antirheumatics/antiinflammatories such as indomethacin, diclofenac or etofenamate" (column 3, lines 1-9, and Claims 9-12 in column 6, lines 16-28).

Again, the medicinal component of the present invention is a specific combination of a local anesthetic and a nonsteroidal antiphlogistic analgesic agent. Moreover, the specific claimed combination of the local anesthetic which is selected from tetracaine, procaine, dibucaine, benzocaine, xylocaine or pharmaceutically acceptable salts thereof, and the nonsteroidal antiphlogistic analgesic agent which is selected from ketoprofen, piroxicam,

felbinac, bufexamac, suprofen, flurbiprofen, ibuprofen or pharmaceutically acceptable salts thereof is ***not*** described in US '112.

The Examiner appears to rely on the position that it is *prima facie* obvious to combine two compositions, each of which is known to be useful for the same purpose, such as analgesics and anesthetics, together for relief of pain.

However, even if arbitrarily selecting any two compositions each of which is useful for relief of pain, the remarkable effect of the present invention cannot be expected. In this regard, it would not have been obvious for one of ordinary skill in the art to determine from the teaching of US '363 and US '874 that an external skin patch of the present invention having a drug reservoir layer which comprises an adhesive gel base containing 20-60% by weight of water and medicinal components containing a specific combination of a local anesthetic and a nonsteroidal antiphlogistic analgesic agent can achieve a superior pain relief effect as evidenced by the Akazawa Declaration and the Nozaki Declaration. The Akazawa Declaration is discussed above.

The Nozaki Declaration describes experiments using Comparative Examples 5-8 and a Test Example 2. This Declaration provides proof that combinations other than the local anesthetic and the nonsteroidal antiphlogistic analgesic agent of the present invention cannot achieve a remarkable pain relief effect.

In particular, the Nozaki Declaration includes a comparison of the claimed combination with the following prior art compositions:

(a) two analgesics -- diclofenac and indomethacin for Comparative Example 5;

(b) two anesthetics --lidocaine and xylocaine for Comparative Example 6;

(c) mixture of an analgesic and a steroidal anti-inflammatory agent -- diclofenac and betamethasone valerate for Comparative Example 7;

(d) two analgesics -- diclofenac and aspirin for Comparative Example 8.

Note that the combination of a local anesthetic and other analgesics, such as aspirin or steroidal anti-inflammatory analgesic agents cannot achieve an unexpectedly superior pain relief effect (see Comparative Examples 7-8).

Further, the pharmaceutical formulation of US '112 is only in a semi-solid phase, specifically in a cream, emulsion, gel, suspension or ointment form, and topical individual doses of the pharmaceutical formulation are situated in individual containers of a molded body.

On the other hand, the pharmaceutical formulation of the present invention is an ***external skin patch*** comprising a substrate and a drug reservoir layer which comprises an adhesive gel base comprising a water soluble polymeric material, a crosslinking agent selected from the group consisting of aluminum compounds, water and a humectant selected from the group consisting of polyhydric alcohols, saccharides and superabsorbent resins. As such, the claimed external skin patch is in an essentially different field of art from the pharmaceutical formulation of US '112. US '112 discloses nothing about an external skin patch of the present invention as a pharmaceutical formulation. Also, the technical background of US '112 is entirely different from the present invention.

The Examiner indicates that US '874 discloses a percutaneous preparation comprising a water soluble polymer, humectants such as polyhydric alcohol, water and a crosslinking agent such as aluminum compounds. US '874 also teaches that the examples of a drug comprising in the preparation include some non-steroidal ant-inflammatory analgesic agents and local anesthetics. However, US '874 mentions nothing about the specific combination of a local anesthetic and a nonsteroidal antiphlogistic analgesic agent of the present invention.

Moreover, the cited patents lack a motivation/suggestion to combine and/or modify the prior art teachings to arrive at the claimed invention. US '874 fails to teach anything about the pharmaceutical formulation as described in US '112. Likewise, US '112 does not teach anything about such preparations as described in US '874. Accordingly, there is no motivation to apply the drug of US '112 to the preparation of US '874, or to apply the preparation of US '874 to the pharmaceutical formulation of US '112.

Therefore, it would ***not*** have been obvious for one of ordinary skill in the art to determine from the teachings of US '112 and US '874 that the specific combination of a local anesthetic and a nonsteroidal antiphlogistic analgesic agent of the present invention can achieve a superior pain relief effect.

Thus, US '112 and US '874 fail to disclose or suggest that the specific combination of a local anesthetic and a nonsteroidal antiphlogistic analgesic agent of the present invention can achieve a superior pain relief effect. These patents also lack the requisite motivation/suggestion to combine their teachings to arrive at the claimed invention.

For these reasons, the rejection of independent claim 7 under 35 U.S.C. § 103(a) as obvious over US '112 and US '874 is untenable and should be reversed.

ii. Dependent Claims 8-11

Again, the inventions of dependent claims 8-11 relate to the external skin patch of claim 7, but further containing a specific local anesthetic and a specific nonsteroidal antiphlogistic analgesic agent in certain amounts.

These claims are also novel and unobvious over the prior art, since, as discussed above, the prior art fails to disclose or suggest the water content and the specific combinations of medicinal agents of the claimed skin patch as claimed.

Thus, in view of above discussion regarding independent claim 7, the rejection of dependent claims 8-11 under 35 U.S.C. § 103(a) as obvious over US '363 and US '874 is also untenable and should be reversed.

VIII. CONCLUSION

For the foregoing reasons, claims 7–11 are novel and unobvious over the cited prior art. Thus, reversal of the final rejections is respectfully requested.

Attached herewith are a Claims Appendix, an Evidence Appendix, and a Related Proceedings Appendix.

Respectfully submitted,

Keiko YAMASAKI et al.

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CLAIMS APPENDIX:

1-6. (Canceled)

7. (Appealed) An external skin patch, comprising a substrate and a drug reservoir layer coated on the substrate, in which the drug reservoir layer comprises (1) an adhesive gel base which comprises a water soluble polymeric material, a crosslinking agent, 20 to 60% by weight of water based upon the total weight of the adhesive gel base and a humectant as essential components, together with (2) medicinal components comprising a local anesthetic and a nonsteroidal antiphlogistic analgesic agent, wherein the crosslinking agent is selected from the group consisting of aluminum compounds, wherein the humectant is selected from the group consisting of polyhydric alcohols, saccharides and superabsorbent resins, wherein the local anesthetic is selected from the group consisting of tetracaine, procaine, dibucaine, benzocaine, xylocaine, and pharmaceutically acceptable salts thereof, and wherein the nonsteroidal antiphlogistic analgesic agent is selected from the group consisting ketoprofen, piroxicam, felbinac, bufexamac, suprofen, flurbiprofen, ibuprofen, and pharmaceutically acceptable salts thereof.

8. (Appealed) The external skin patch according to claim 7, wherein the local anesthetic is present in an amount of 0.1-50% by weight.

9. (Appealed) The external skin patch according to claim 7, wherein the nonsteroidal antiphlogistic analgesic agent is present in an amount of 0.05-10% by weight.

10. (Appealed) The external skin patch according to claim 7, wherein the local anesthetic is selected from the group consisting of benzocaine and a pharmaceutically acceptable salt thereof.

11. (Appealed) The external skin patch according to claim 7, wherein the nonsteroidal antiphlogistic analgesic agent is selected from the group consisting of ketoprofen, felbinac, flurbiprofen, and a pharmaceutically acceptable salt thereof.

12. (Canceled)

EVIDENCE APPENDIX:

1. Drug Discovery and Evaluation, p 384-385, p406-409, Vogel et al. eds. (1997) is attached herewith and is being submitted to respond to the arguments first raised in the Advisory Action.

2. The Declaration under 37 C.F.R. § 1.132 by Mitsuji Akazawa is attached herewith and was submitted in the after final response filed December 30, 2004, and was considered and entered in the record by the Examiner in the Advisory Action of January 19, 2005.

3. The Declaration under 37 C.F.R. § 1.132 by Keiji Nozaki is attached herewith and was submitted in the response filed May 20, 2004, and was considered and entered in the record by the Examiner in the final Office Action of June 30, 2004.

RELATED PROCEEDINGS APPENDIX:

None

Sir:

I, Keiji NOZAKI, declare as follows:

I. IDENTIFICATION OF DECLARANT

I am employed by TEIKOKU SEIYAKU CO., LTD and hold the position of associate manager of New Product Planning Department.

My educational background is the following:

Graduated from Kyushu University

Bachelor's Degree in Engineering

II. DETAILS OF EXPERIMENTS

I have conducted personally or under my direction and control the following experiments:

Comparative Example 5

An external skin patch was prepared in the same production process employed in Example 1 except that 0.5 parts by weight of indomethacin was used instead of lidocaine. The total amount of the drug-containing base was fixed to 100 parts by weight by adjusting the amount of purified water. The amount of indomethacin was determined according to the amount used generally in Japan as an effective amount of the medicine.

Comparative Example 6

An external skin patch was prepared in the same production process employed in Example 1 except that 5 parts by weight of xylocaine was used instead of sodium diclofenac. The total amount of the drug-containing base was fixed to 100 parts by weight by adjusting the amount of purified water. The amount of xylocaine was determined according to the amount used generally in Japan as an effective amount of the medicine.

Comparative Example 7

An external skin patch was prepared in the same production process employed in Example 1 except that 0.1 parts by weight of betamethasone valerate which was one of the steroidal ant-inflammatory agents cited

in US 5,725,874, column 2, line 58-59 was used instead of lidocaine. The total amount of the drug-containing base was fixed to 100 parts by weight by adjusting the amount of purified water. The amount of betamethasone valerate was determined according to the amount used generally in Japan as an effective amount of the medicine.

Comparative Example 8

An external skin patch was prepared in the same production process employed in Example 1 except that 5.0 parts by weight of aspirin which was one of the general analgesic agents cited in US 5,725,874, column 3, line 13 was used instead of lidocaine. The total amount of the drug-containing base was fixed to 100 parts by weight by adjusting the amount of purified water. Since aspirin is rarely used as a drug for an external preparation, the amount of aspirin in this comparative example was determined in consideration of the circumstance of drug preparation.

Test Example 2

The external skin patches obtained in Example 1 and Comparative Examples 5 to 8 were administered randomly to 10 volunteers a group (total 50 persons) each having low back pain (i.e. plastered on the affected part) and an organoleptic examination was carried out. The duration of the administration was 12 hours a day and the test was carried out for 7 days. After the test, volunteers rated the results on a 1-to-4 scale ("complete remission", "effective", "unchanged" and "aggravation".) The results are given in Table 8.

Table 8

	Example 1	Comparative Example 5	Comparative Example 6	Comparative Example 7	Comparative Example 8
Complete Remission	7	2	1	1	1
Effective	3	5	5	4	5
Unchanged	0	2	4	5	4
Aggravation	0	1	0	0	0

As shown above, the amelioration ratio (effective or higher) of the external skin patches of Examples 1 and Comparative Examples 5 to 7 after 1 week was respectively 100% (10/10), 70% (7/10), 60% (6/10), 50% (5/10), and 60% (6/10), and the ratio of the Complete Remission was respectively 70% (7/10), 20% (2/10), 10% (1/10), 10% (1/10), and 10% (1/10).

This shows that the external skin patch using the combination of the particular local anesthetic and the nonsteroidal antiphlogistic analgesic agent of the present invention (Example 1) achieves superior pain relief effect compared to the external skin patches using any other combination of the compounds which are known to be useful for relief pain (Comparative Examples 5 - 8).

Thus, it is not obvious to the skilled artisan that using the specific combination of the local anesthetic and the nonsteroidal antiphlogistic analgesic agent of the present invention can only achieve a remarkable effect and any other random combination of drugs can not be achieved the same.

III. CONCLUSION

The foregoing experiments demonstrate that the external skin patch according to the claimed invention using a combination of local anesthetic and a non-steroidal antiphlogistic analgesic agent achieves an unexpectedly superior pain relief effect compared to an external skin patch according to the prior art using any other combination of compounds known to be effective for pain relief.

IV. VERIFICATION CLAUSE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 15/4/2004 Signature: Keiji Nozaki

Sir:

I, Mitsuji AKAZAWA, declare as follows:

I. IDENTIFICATION OF DECLARANT

I am employed by TEIKOKU SEIYAKU CO., LTD and hold the position of associate director of New Product Planning Department.

My educational background is the following:

Graduated from Tokushima University

Bachelor's Degree in Engineering

II. DETAILS OF EXPERIMENTS

I have conducted personally or under my direction and control the following experiments:

1. Preparation of a dosing composition

1) Reagent; 10% suspension of brewer's yeast (suspended in saline so that the concentration of brewer's yeast becomes 10%).

2) Test substance; a patch cut to a 3×4 cm square.

2. Animal used

Rats of Wistar strain (male, 5 weeks old)

3. Test Procedure

(1) Stimulus of pressure was added to a right hind paw of each rat by using a measuring device of pain threshold, and the pain threshold was measured for each rat by determining "biting", "screaming" and "drawing the hinder leg away" as a larvate pain reaction.

Individuals showing the pain threshold of 50 to 61 mmHg were selected and divided into groups consisting of 15 individuals per group.

(2) Each test substance was topically applied and fixed by paper tapes to the right hind paws of the rats. In addition, same operations were conducted except for applying no test substances for a non-treated group.

(3) The test substances were removed after 4 hours of application of the test substances. Then, inflammation was induced by injecting 0.1 ml of brewer's yeast suspension under the skin of a pad of the right

paw.

(4) Pain threshold was measured for each rat 3 hours after the induction of inflammation, whereby the ratio of the pain threshold was obtained according to the following formula (1) and then the rate of increase of the pain threshold for the group dosed test substances based upon that for the non-treated group was calculated according to the following formula (2).

Formula (1); $A = A_1 / A_0$

In the formula (1), A is the ratio of the pain threshold, A_1 is the pain threshold after the brewer's yeast injection and A_0 is the pain threshold before the brewer's yeast injection.

Formula (2); $B\ (\%) = (B_1 - B_0) / B_0 \times 100$

In the formula (2), B is the rate of increase of the pain threshold (%), B_1 is the ratio of the pain threshold for the group dosed each test substance and B_0 is the ratio of the pain threshold for the non-treated group.

4. Examples

1) Example (A) wherein the content of water is 60% by weight and Example (B) wherein the content of water is 20% by weight:

An external skin patch was prepared in the same production process employed in Example 1 except that the amount of each component was shown in Table I.

Table I

	Example(A)	Example(B)
Water content	60%	20%
Ingredients		
Sodium Diclofenac	1	1
Lidocaine	5	5
Propylene Glycol	10	10
N-methyl-2-pyrroridone	5	5
70% Sorbitol solution *	3.1	60.3
Sodium Polyacrylic Acid	5	5
Carboxymethylcellulose Sodium	4	4

Dry Aluminum Hydroxide Gel	0.3	0.3
Tartaric Acid	2.5	2.5
Kaolin	5	5
Water	59.1	1.9

*: 70% sorbitol solution contains 30% of water by weight.

2) Comparative Example (A) of a skin patch with substantially water-free:

An external skin patch which is a substantially water-free film was prepared in the same production process employed in Example 8 of US '363 except that reagents and the concentration of each reagent were same as the above Example (A) and (B) as shown in Table II.

Table II

Ingredients	Comparative example (A)
Karaya Gum	40
Polyvinylpyrrolidone	20
Oleic Acid	34
Sodium Dicrofenac	1
Lidocaine	5
Ethanol	(100)

5. Result :

Table III

Examples	Ratio of Pain Threshold	Increase of Pain Threshold (%)
Control (Non-treated)	0.43 ± 0.02	—
Example (A)	0.67 ± 0.01**	55.8
Example (B)	0.66 ± 0.02**	53.5
Comparative Example (A)	0.52 ± 0.02	20.9

Tukey's multiple range test: **; $p<0.01$ (vs. Control)

The above Examples (A), (B) and Comparative Example show that the external skin patches wherein the content of water is 20 to 60% by weight based upon the total weight of the adhesive gel base can achieve superior pain relief effect compared to the external skin patches wherein the content of water is less than 10% by weight as US'363.

III. CONCLUSION

The foregoing experiments demonstrate that the external skin patch according to the claimed invention wherein the content of water is 20 to 60% by weight achieves an unexpectedly superior pain relief effect compared to an external skin patch according to the prior art wherein the content of water is less than 10% by weight.

IV. VERIFICATION CLAUSE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12/21/2004 Signature: [signature]

H. Gerhard Vogel • Wolfgang H. Vogel (Eds.)

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H.2.0.3 Pain in inflamed tissue (RANDALL-SELITTO-test)

PURPOSE AND RATIONALE

This method for measuring analgesic activity is based on the principle that inflammation increases the sensitivity to pain and that this sensitivity is susceptible to modification by analgesics. Inflammation decreases the pain reaction threshold and this low pain reaction threshold is readily elevated by non-narcotic analgesics of the salicylate-amidopyrine type as well as by the narcotic analgesics. Brewers yeast has been used as an inducer for inflammation which increases pain after pressure.

PROCEDURE

Groups of male Wistar rats (130 to 175 g) are used. Only for oral testing the animals are starved 18 to 24 hours prior to administration. Otherwise, the route of administration can be intraperitoneal or subcutaneous. To induce inflammation, 0.1 ml of a 20% suspension of Brewer's yeast in distilled water is injected subcutaneously into the plantar surface of the left hind paw of the rat. Three hours later, pressure is applied through a tip to the plantar surface of the rat's foot at a constant rate by a special apparatus to the point at which the animal struggles, squeals or attempts to bite. The apparatus being used has been modified by various authors such as using the Analgy Meter (Ugo Basile, Apparatus for Biological Research, Milan, Italy). Each animal is tested for its control pain threshold. Any animal with a control pain threshold greater than 80 g is eliminated and replaced.

For a time response, groups of at least 7 animals are used, four groups for the agent to be tested and one for the vehicle control. The tests are done at 15 minutes intervals after subcutaneous administration and at 30 minutes intervals after oral administration for any change in pain threshold. The interval of time which indicates the greatest increase in pain threshold is regarded as the peak time.

A dose range is obtained in the same manner as the time response. The drug to be tested is administered in a randomized manner. The pain threshold is recorded at time zero and again at the determined peak time.

EVALUATION

The mean applied force is determined for each time interval tested. The percentage increase in pain threshold is calculated by subtracting the applied force of the vehicle control from the applied force of the drug group which is divided by the applied force of the vehicle control in order to give the percentage of increase in pain threshold of the drug group. Doses of 50 mg/kg s.c. Na salicylate, 50 mg/kg amidopyrine, 3 mg/ kg s.c. morphine, 12.5 mg/kg s.c. codeine or pethidine have been found to be effective.

CRITICAL ASSESSMENT OF THE METHOD

The method originally described by RANDALL and SELITTO has been used by many investigators and has been proven to detect central analgesics as well as peripheral analgesics. Peripherally acting analgesics such as the nonsteroidal anti-inflammatory drugs increase only the threshold of the inflamed paw, whereas opiate analgesics increase also the threshold of the intact paw (Dubinsky et al 1987). In most modifications, the assay has a shallow dose-response curve. Nevertheless, the ED_{50} values of nonsteroidal anti-inflammatory drugs in this test showed a good correlation with human doses (Romer 1980).

MODIFICATIONS OF THE METHOD

The test has been modified by various authors. In some instances the pressure on the inflamed paw has been omitted. Instead the animals were allowed to walk on a metal grid. The gait of the animals is assessed by an observer using a scoring system:

0 = three-legged gait
0.5 = marked limping
1 = normal gait.

The scores are transformed into percent analgesia.

Other noxious stimuli were used to induce inflammation and hyperalgesia, such as carrageenin (Winter

et al 1962), Freund's adjuvant or prostaglandin E_2 (Ferreira et al 1978 a).

Vinegar et al (1990) injected 0.1 ml of 0.25% solution of trypsin into the subplantar region and applied the load force 60 min later. They found a biphasic hyperalgesia and relatively low ED_{50} values for central and peripheral analgesics.

Technically, the method has been improved by several authors, such as Takesue et al (1969). Chipkin et al (1983) modified the test by decreasing the rate of acceleration of the noxious stimulus (mechanical pressure) on the inflamed paw from 20 to 12.5 mm Hg/sec and an extension of the cut-off time from 15 to 60 sec. This modification is claimed to discriminate analgesics active against mild to severe clinical pain (narcotic-like) from those only useful against mild to moderate pain (non-narcotic-like).

Central and peripheral analgesic action of aspirin-like drugs has been studied with a modification of the Randall-Selitto method applying constant pressure to the rat's paw by Ferreira et al (1978 b).

A modification of an analgesia meter for paw pressure antinociceptive testing in neonatal rats was described by Kitchen (1984).

Learning and retention has been tested in rats by Greindl and Preat (1976) inducing pain by a light quantifiable pressure applied to the normal hind paw.

Hargreaves et al (1988) described a sensitive method for measuring thermal nociception in cutaneous hyperalgesia in rats. One paw was injected with 0.1 ml carrageenan solution, the other paw with saline. The rats were placed in chambers with glass floor and radiant heat was directed to the paws. A photoelectric cell detected the light reflected from the paw and turned off the radiant heat when paw movement interrupted the reflected light.

Perkins et al (1993) described hyperalgesia after injection of 100 µl of Freund's adjuvant into the knee of anesthetized rats. After 64–70 h the animal was placed with each hind paw on a pressure transducer and a downward force was exerted until the uninjected leg was bearing 100 g. At this point animals were less tolerant to a load on the injected leg, indicating a hyperalgesic response.

Ferreira et al (1993 a, 1993 b) induced hyperalgesia by intraplantar injection in the hindpaw of rats of various agents, e.g., bradykinin, carrageenin, LPS, PGE_2, dopamine, TNFα, IL-1β, IL-6 and IL-8. A constant pressure of 20 mm Hg was applied to the hind paws and discontinued when the rats presented a typical freezing reaction.

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Maloff et al (1989) injected 20 µl of interleukin-1 solution into the left ear of mice and found a dose-dependent increase of ear thickness and myeloperoxidase activity which reached the maximum after 24 hours. These effects were reduced by high doses of glucocorticoids but not by nonsteroidal anti-inflammatory drugs.

Chang et al (1987) applied 4 µg tetradecanoyl phorbol acetate and test drugs dissolved in acetone to the right ear of mice. Ear edema was calculated by subtracting the thickness of the left ear (vehicle control) from the right ear (treated ear).

Topical application of arachidonic acid to mouse ear has become a widely used test (Young et al 1983, 1984, Opas et al 1985, Crummey et al 1987, Tomchek et al 1991) One mg arachidonic acid is applied to the right ear of mice and vehicle to the left ear of each animal. Drugs are topically applied in acetone to the ear 30 min prior to the arachidonic acid application. Ear swelling was measured using a caliper one hour after arachidonic acid.

Griswold et al (1995) induced inflammation in mice by local application of arachidonic acid or phorbol ester. Besides ear thickness, myeloperoxidase and DNA content was measured.

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H.3.2.1.6 Paw edema

PURPOSE AND RATIONALE

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin, Aerosil(R), sulfated polysaccharides like carrageenin or naphthoylheparamine. The effect can be measured in several ways. The hind limb can be dissected at the talocrural joint and weighed. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume of the treated animals is compared to the controls. Many methods have been described how to measure the paw volume by simple and less accurate and by more sophisticated electronically devised methods. The value of the assessment is less dependent on the apparatus but much more on the irritant being chosen. Some irritants induce only a short lasting inflammation whereas other irritants cause the paw edema to continue over more than 24 hours.

PROCEDURE

Male or female Sprague-Dawley rats with a body weight between 100 and 150 g are used. The animals are starved overnight. To insure uniform hydration, the rats receive 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, again 3 and 6 hours, and eventually 24 hours after challenge.

Apparatus

Various devices have been developed for plethysmography of the paw. Winter et al. (1963) used mercury for immersion of the paw. A more sophisticated apparatus has been described by Hofrichter et al (1969). Alpermann and Magerkurth (1972) described an apparatus based on the principle of transforming the volume being increased by immersion of the paw into a proportional voltage using a pressure transducer. Webb and Griswold (1984) reported a sensitive method of measuring mouse paw volume by interfacing a Mettler DeltaRange top-loading balance with a microcomputer. Several authors used a commercially available plethysmometer from Ugo Basile, Varese, Italy (Damas and Remacle-Volon 1992, Braga da Motta et al 1994, Legat et al 1994, Griesbacher et al 1994).

EVALUATION

The increase of paw volume after 3 or 6 hours is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals give some hints for the duration of the anti-inflammatory effect. A dose- response curve is run for active drugs and ED_{50} values can be determined.

MODIFICATIONS

Many agents can be used as irritants to induce paw edema in rats or mice. These are:

- 0.05 ml undiluted fresh egg white (Randall and Baruth 1976)
- 0.1 ml of 1% ovalbumin solution (Turner 1965)
- 0.1 ml of 1% formalin (Turner 1965)
- 0.1 ml of 0.2% carrageenan solution (Schönhöfer 1967)
- 0.1 ml of 1 to 3% dextran solution (Turner 1965)
- 0.1 ml of 2.5% brewer's yeast powder suspension (Tsumuri et al 1986)
- 0.1 ml of 0.5% ß-naphthoylheparamine solution (Peterfalvi et al 1966)
- 0.1 ml of 0.1% trypsin solution (Kalbhen and Smalla 1977)
- 0.1 ml of 0.1% collagenase solution (Souza Pinto et al 1995)
- 0.1 ml of 0.1% solution of collagenase from Clostridium histolyticum (Legat et al 1994)
- 0.1 ml of solution of 100 IU hyaluronidase (Dewes 1955, Kalbhen and Smalla 1977).
- 0.1 ml of complete Freund's adjuvant
- 0.05 ml of 0.02% serotonin solution (Kalbhen and Smalla 1977)
- 0.1 ml of 0.005% bradykinin solution (Damas and Remacle-Volon 1992)
- 0.1 ml of 0.1 mg/ml prostaglandin E2 (Nikolov et al (1978)
- 0.1 ml of 2.0 µg/ml prostaglandin E2 (repeated injections, Willis and Cornelsen 1973)
- 0.1 ml of 1% concanavalin A solution (Lewis et al 1976)
- 0.1 ml of 2.5% suspension of Aerosil ®
- 0.1 ml of 5% suspension of kaolin (Lorenz 1961, Wagner-Jauregg et al 1962)
- 0.05 ml of bentonite gel (Marek 1980)
- 0.1 ml of nystatin 15,000 units (Schiatti et al 1970, Arrigoni-Martelli et al 1971)
- 0.1 ml of 1% phytohaemagglutinin-P solution (Lewis et al 1976)
- 0.01 ml of 0.5% adriamycin (mouse paw) (Siegel et al 1980)
- 0.1 ml of 0.001–0.1% solutions of various phospholipases A2 (Cirino et al 1989)
- 0.1 ml of 0.1% Zymosan solution (Gemmell et al 1979)
- 0.1 ml of 0.05% anti-IgG solution (Gemmell et al 1979)
- 0.1 ml of 2.5% mustard powder suspension (Tsumuri et al 1986)
- 0.1 ml of solution containing 1 unit of cobra venom factor (Leyck and Parnham 1990)
- 0.05 ml of 0.02–0.2% sonic extract from Porphyromonas gingivalis (Griesbacher et al 1994)

The edema induced by the various irritants lasts for different times such as a few hours after serotonin and up to 2 days after Aerosil® or after kaolin. These irritants therefore are suitable to study not only the degree but also the duration of the anti-inflammatory action.

Standards

Depending on the irritant steroidal and nonsteroidal anti-inflammatory drugs have a pronounced effect in the paw edema test. With carrageenan as irritant doses of 50 to 100 mg/kg phenylbutazone p.o. have been found to be effective.

CRITICAL ASSESSMENT OF THE METHOD

The paw edema method has been used by many investigators and has been proven to be suitable for screening purposes as well as for more in depth evaluations. Dependent on the irritant steroidal and nonsteroidal anti-inflammatory drugs, antihistaminics and also, to a lesser degree, serotonin antagonists are active in the paw edema tests. Since so many different irritants have been used by the various investigators the results are often difficult to compare.

FURTHER MODIFICATIONS OF THE METHOD

Besides paw volume Shirota et al (1984) determined the surface temperature of the inflamed paw in rats using a special cage with rolling rods.

Brooks et al (1991) used anesthetized dogs and demonstrated that a significant inflammatory response can be elicited in the dog paw by subcutaneous injection of carrageenin. The increase in paw volume can be quantitatively measured as a pressure change recorded via a water-filled balloon fixed against the paw with nonexpandable tape. Effective doses of nonsteroidal antiinflammatory drugs were closer to human therapeutic doses in dogs than in rats.

Oyanagui and Sato (1991) described an ischemic paw assay in mice. A commercial rubber ring (1×1 mm, d = 42 mm) was bound 14 times to the right hind leg of mice just above the articulation. After 20 min of ischemia, the rubber was cut off with scissors. Paw swelling was measured after an other 20 min of natural blood recirculation.

Wirth et al (1992) described a thermic edema which was induced in anesthetized Sprague-Dawley rats by immersing paws of the right and left hindlimb into water of 55°C. Immediately thereafter, the rats received the test drug (the bradykinin antagonist Hoe 140) intravenously. Paw volume was measured at regular intervals by plethysmography.

Braga da Motta et al (1994) described drug modulation of antigen-induced paw edema in guinea-pigs. Male short-haired guinea pigs weighing 250–350 g received on day 0 a single dorsal s.c. injection of 1 ml of phosphate buffered saline containing 20 µg of ovalbulmin, dispersed in 1 mg $Al(OH)_3$. The animals were boosted with a similar injection of antigen on days 14, 21, and 28. Thirty five days after the first injection of antigen or $Al(OH)_3$, the animals received an intraplantar injection of 0.5, 5, 50, or 200 µg ovalbumin, diluted in 100 µl of phosphate buffered saline. Edema was measured 2, 4, 6, 8, 24, and 48 h after the challenge.

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H.3.2.1.7 Pleurisy test

PURPOSE AND RATIONALE

Pleurisy is a well known phenomenon of exudative inflammation in man. In experimental animals pleurisy can be induced by several irritants, such as histamine, bradykinin, prostaglandins, mast cell degranulators, dextran, enzymes, antigens, microbes, and nonspecific irritants, like turpentine and carrageenan (Survey by DeBrito 1989). Carrageenan-induced pleurisy in rats is considered to be an excellent acute inflammatory model in which fluid extravasation, leukocyte migration and the various biochemical parameters involved in the inflammatory response can be measured easily in the exudate.

PROCEDURE

Male Sprague-Dawley rats weighing 220–260 g are used. The animal is lightly anaesthetized with ether, placed on its back and the hair from skin over the ribs of the right side is removed using animal clippers. The region is swabbed with alcohol. A small incision is made into the skin under the right arm between the seventh and eighth rib. The wound is opened and a further shallow incision is made into the exposed intercostal muscle. 0.1 ml of 2% carrageenin solution is injected into the pleural cavity through this incision. The injection needs to be made swiftly to avoid the risk of injuring the lung. The wound is closed with a Michel clip.

One hour before carrageenin injection and 24 and 48 hours thereafter, groups of 10 rats are treated with the standard or the test compound subcutaneously or orally. A control group receives only the vehicle of medication. The animals are sacrificed 72 hours after carrageenin injection by ether inhalation. The animal is pinned on a dissection board with the forelimbs fully extended. An incision in the skin over the xiphosternal cartilage is made to free the cartilage from overlying connective tissue. The cartilage is lifted with a forceps and a small cut is made with scissors in the body wall below to gain access into the pleural cavity. One ml of heparinized Hank's solution is injected into the pleural cavity through this cut. The